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Research Note

Experimental Infections with the Tasmanian Isolate of *Trichinella pseudospiralis* Using a Non-enzymatic Recovery Technique

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ABSTRACT: Laboratory rats and mice, cats, brushtail possums (*Trichosurus vulpecula*), and 2 species of raptor (marsh harrier, *Circus aeruginosus,* and brown falcon, *Falco berigora*) were either infected orally with larvae of *Trichinella pseudospiralis* isolated by a non-enzymatic technique or by feeding infected muscle tissue. Muscle from a naturally infected Tasmanian devil (*Sarcophilus harrisii*) and an eastern quoll (*Dasyurus viverrinus*) resulted in infections in cats, rats, and marsh harriers. Similarly, larvae derived from feline muscle were infective for mice and a brown falcon. Infected muscle tissue from marsh harriers was also infective for the same species. The reproductive capacity index (RCI) for rats fed larvae from an eastern quoll was 34.5, whereas the RCI for mice infected with larvae derived from a cat was 31.6.

KEY WORDS: *Trichinella pseudospiralis,* experimental infections, non-enzymatic digestion.

Following the discovery of *Trichinella pseudospiralis* Garkavi, 1972, on the island of Tasmania in 1987, experimental infections were conducted at the Australian Animal Health Laboratory (AAHL) in Geelong, Victoria (Obendorf et al., 1990). Those studies demonstrated that laboratory rats and mice, pigs, and chickens were susceptible to infection; however, the reproductive capacity index (RCI; the ratio of larvae recovered from tissues of the experimentally infected individuals/number of larvae fed) in rodents and chickens was low. Additional rodent infections using muscle larvae liberated by rapid digestion of infected meats in a 1% pepsin/0.5% concentrated hydrochloric acid solution were unsuccessful, whereas brushtail possums (*Trichosurus vulpecula*) fed freshly minced muscle from the same source became infected (unpubl. data). Because the means for establishing these experimental infections were different, no worthwhile conclusions could be drawn about the relative susceptibility of placental and marsupial mammals.

Recent evidence indicates that when *T. pseudospiralis* larvae are exposed twice to low pH and pepsin, once during isolation by the conventional enzymatic digestion method and a second time when larvae are inoculated into the stomach of a host animal, their infectivity declines dramatically (Stewart and Deford, 1989; Stewart et al., 1990). In light of these reports, further experimental infections of placental mammals, brushtail possums, and birds of prey were attempted. The primary aims were to ascertain (1) whether or not the brushtail possum (a primarily herbivorous marsupial that will take other food, including meat) and laboratory rodents (rats and mice) could be infected with the Tasmanian isolate of *T. pseudospiralis* using larvae recovered by the non-enzymatic method of Stewart and Deford (1989), (2) whether or not the introduced feral cat *Felis catus* is susceptible to infection, and (3) whether or not bird-to-bird transmission is possible.

Trichinella pseudospiralis larvae were recovered from muscle tissues of two naturally infected dasyurid marsupials, a Tasmanian devil, *Sarcophilus harrisii,* and an eastern quoll, *Dasyurus viverrinus,* according to the method of Stewart and Deford (1989). Larvae were used to infect 4 wild-caught brushtail possums (380 larvae each) and 12 8-wk-old laboratory-reared rats (200 larvae each). The remaining muscle tissue was fed to 2 unweaned 6-wk-old kittens (*Felis catus*) and 2 wild-caught marsh harriers (*Circus aeruginosus*). Forty-five days postinfection (DPI), the marsh harriers were killed and muscle tissues fed to another marsh harrier. One kitten was killed at 8 DPI; the small intestine was examined for the presence of adult *T. pseudospiralis.* Larvae, recovered from the other cat (60 DPI), were fed orally to 6 8-wk-old mice (28 larvae/mouse). Two infected mice were killed at 60 DPI and fed, as whole carcasses, to a brown falcon (*Falco berigora*).

The brushtail possums were killed at between 52 and 82 DPI, and 10-g samples of selected muscles were digested for 12 hr in a solution of 1% pepsin and 0.5% concentrated hydrochloric acid. The mice and rats were killed 60 DPI, and

the entire body musculature was digested. Digest fluids were passed through a 53-μm sieve, and the material collected was examined by light microscopy at 40× magnification. Digests of pectoral and limb muscles were also performed on the marsh harrier 45 DPI infected with muscle from the 2 original marsh harriers and on the brown falcon 30 DPI infected with mice.

Irrespective of the source of *T. pseudospiralis* larvae, infections were established in all hosts. Although no RCI for the experimentally infected possums was obtained, the larval recovery per gram from selected muscles was very high (Table 1). Two cats became infected after eating meat from a Tasmanian devil. Eight DPI, adult *T. pseudospiralis* worms were recovered from the small intestine of 1 kitten. Of the worms recovered (n = 168), 53% were in the distal third of the small intestine. Infected rats, each dosed with 200 larvae from an eastern quoll, had an RCI of 34.5 (SD ± 6.8; n = 3). Two marsh harriers fed minced muscle tissue from the same eastern quoll had 0.8 and 2.2 larvae/g in their muscles. Subsequently, when muscle tissue from these harriers was fed to another marsh harrier, 5.6 muscle larvae/g were recovered. Laboratory mice originally infected with larvae derived from a cat had an RCI of 31.6 (SD ± 17.7; n = 4). Infection was also established when mice infected with cat-derived *T. pseudospiralis* larvae were fed to a brown falcon; 18.7 larvae/g were recovered.

These findings suggest that a wide range of hosts is potentially capable of becoming infected with the Tasmanian isolate of *T. pseudospiralis*, and this is in agreement with previous experimental studies using Northern Hemisphere isolates of *T. pseudospiralis* (Garkavi, 1974; Meerovitch and Chadee, 1982; Tomasovicova and Hovorka, 1982; Bober and Dick, 1983).

Rodents were readily infected with the Tasmanian isolate with RCI values exceeding 30. This value is considerably higher than 0.1–3.2 obtained in earlier studies at the AAHL using larvae derived by a rapid pepsin/HCl digestion technique (Obendorf et al., 1990). As demonstrated by Stewart et al. (1990), experimental studies that more closely reflect the natural mode of infection, namely, ingestion of muscle tissues or larvae recovered by a non-enzymatic extraction technique, enhance the infectivity of *T. pseudospiralis*.

No *T. pseudospiralis* infections were detected in a sample of 22 feral cats (Obendorf et al., 1990); however, the study presented here shows that cats are capable of becoming infected with this parasite.

Table 1. Recovery of *Trichinella pseudospiralis* larvae from selected muscles in brushtail possums (*Trichosurus vulpecula*) each dosed with 380 larvae.*

Possum number	DPI	Selected muscles (larvae/g)						
		dia.	int.	mass.	abdo.	quad.	neck	subcut.
#1	52	215	192	106	90	69	96	5
#2	82	102	ND	27	26	25	56	15
#3	56	23	3	16	5	4	14	6
#4	61	444	266	1,278	269	191	638	100

* Abbreviations: DPI = days postinfection; dia. = diaphragm, int. = intercostal, mass. = masseter, abdo. = abdominal, quad. = quadriceps, neck = cervical muscles, and subcut. = subcutaneous muscles; ND = not done.

At least 2 species of carnivorous or carrion-feeding bird (masked owl, *Tyto novaehollandiae*, and marsh harrier) are known to be naturally infected in Tasmania (Obendorf and Clarke, 1992). An obvious limitation with these experimental infections of the birds and possums is not knowing whether or not the 3 marsh harriers, the brown falcon, and the brushtail possums were uninfected prior to these studies.

In these experiments, brushtail possums were readily infected with *Trichinella pseudospiralis*, yet in the survey of Obendorf et al. (1990) infection was detected in only 1 of 145 free-living possums. These differences may reflect the infrequency with which brushtail possums actually feed on infected carcasses.

These experimental infections were conducted using dasyurid carcasses obtained as road kills. The assistance of N. Mooney, Department of Parks, Wildlife and Heritage, in making disabled and injured raptors available is also gratefully acknowledged. I wish to thank particularly Jason Wiersma for caring for these birds after they were fed with infected muscle tissues.

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Research Note

Acanthocephalans from the Orangethroat Darter, *Etheostoma spectabile,* from the Wabash Lowlands

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ABSTRACT: Two species of acanthocephalan parasites infected orangethroat darters collected from a stream in southwestern Indiana. *Acanthocephalus dirus* and *Pomphorhynchus bulbocolli* infected 85 and 15% of the darters examined, respectively. The difference in prevalence may be a function of food item selection patterns of the piscine host with regard to the parasites' intermediate host.

KEY WORDS: *Etheostoma spectabile,* orangethroat darter, *Acanthocephalus dirus, Pomphorhynchus bulbocolli,* Indiana.

The parasites of darters (Pisces: Percidae) have been casually mentioned by authors in the course of life history studies (see review by Page, 1983). However, except for a report by Buckner et al. (1985), little is known about the parasites of the darters inhabiting the Wabash Lowlands of southwestern Indiana. This note presents new information on the parasites infecting the orangethroat darter, *Etheostoma spectabile.*

Twenty orangethroat darters were collected from Road Brook, a first-order tributary of the Wabash River in Posey County, Indiana. Collections were made by seine between 20 December 1990 and 20 February 1991. Darters were preserved in 10% formalin and necropsied within 24 hr of collection. Darters were examined for endoparasites by dissecting through the gastrointestinal tract from the cardiac valve to the anus. Parasites were transferred to alcohol–formalin–acetic acid, stained with Semichon's acetocarmine, and mounted whole in Permount. Food items were quantified and identified to lowest practical taxon. Voucher specimens of *Acanthocephalus dirus* (USNM Helm. Coll. No. 82689) and *Pomphorhynchus bulbocolli* (USNM Helm. Coll. No. 82690) have been placed in the USNM Helminthological Collections, Beltsville, Maryland 20705. Specimens of the orangethroat darter hosts have been placed in the Southern Illinois University Ichthyology Collection (SIUC 20246 and 20247).

Food items of this orangethroat darter population consisted primarily of chironomid larvae (67.1% of total items, 80% freq.) and isopod crustacea (22.5% of total items, 65% freq.). Amphipod crustacea (4.6% of total items, 40% freq.), tricopteran larvae (3.9%, 25% freq), and oligochaete worms (1.7%, 5% freq.) were minor constituents of the diet. Seventeen of the 20 darters examined were parasitized by *Acanthocephalus dirus* Van Cleave, 1931 (85% prevalence), with

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